PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/005239	15.05.2004	06.06.2003

International Patent Classification (IPC) or both national classification and IPC
C12N9/90, C12P41/00, C12Q1/533, C12Q1/34, C12N15/61, C12N5/10, C12N15/63

Applicant
DEGUSSA AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/005239

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 5-14

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 5-14

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-4

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-4
	No:	Claims	NONE
Inventive step (IS)	Yes:	Claims	NONE
	No:	Claims	1-4
Industrial applicability (IA)	Yes:	Claims	1-4
	No:	Claims	NONE

2. Citations and explanations

   see separate sheet

Re Item III

1. According to Rule 66.1(e) PCT, claims relating to inventions in respect of which no international search report has been established need not be the subject of international preliminary examination. As the subject-matter of claims 5-14 has not been searched (see BOX I of the International Search Report), no preliminary examination has been carried out for these claims.

Re Item IV

1. The application lacks unity as contravening the requirements of Rule 13 PCT. Rule 13.1 PCT states that for unity of invention to be present, all subject-matter should be linked by a single general inventive concept. Rule 13.2 PCT stipulates that where a group of inventions is claimed the requirement of unity shall be fulfilled only where there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. "Special" technical features are those features that define a contribution which each of the inventions makes over the prior art.

2. Five different (groups of) potential inventions have been recognised (see list). The common concept (technical relationship) linking these Groups together and covering the whole of the claimed subject-matter, with due consideration paid to the content of the description section, is the hydantoin racemase activity. Said enzymatic activity was known from the prior art (see abstracts of the first two document cited in the search report) and thus cannot define a contribution over the prior art. No "corresponding" special technical features could be identified either. Since no other feature could be identified neither in the description nor in the claims that could be considered a "special" technical feature in the sense of Rule 13.2 PCT, each group must be regarded as a separate group of inventions.

3. Consequently, the different inventions lacking a common inventive concept were formulated as different subjects (the Applicant's attention is drawn to the fact that the use of the term "invention" here in no way implies recognition of an inventive step for the subject-matter of any group).

   A complete search could be performed with relatively little effort: due to the lack of common technical features independent searches have to be carried out for each of the inventions. The search thus has been limited to the first-claimed invention and additional fees were required for each additional subject to be searched.

4. As the Applicant did not pay additional fees, the international search and written opinion are drawn up on the first recognized invention.

Re Item V

1. Present claims 1 and 4 relate to processes defined (inter alia) by reference to the following parameters:
   Claim 1: section b) a hydantoin racemase which has a slower conversion rate compared to the hydantoinase under a).
   Claim 4: the ratio of the rate constants of the hydantoinase to the hydantoin racemase is bigger than 2.

   The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible.

   Furthermore, present claims 1-4 relate to a process defined by reference to desirable properties, namely fulfilling the functional statements recited in claims 1 and 4. The claims cover all processes having these properties, whereas the application does not provides sufficient support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for said processes. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

   Even further, claims 1 and 4 are further unclear. The screening process is apparently intended to identify new hydantoin racemases. According to claim 1b and claim 4, the racemase need to have a slower conversion rate than the hydantoinase. Thus if appears contradictory that the conversion rate need to be known before the screening has been made.

   Consequently, the search had been restricted to hydantoinases and racemases of the prior art even if their conversion rates (rate constants) were not disclosed in said documents.
2. The document numbering corresponds to the order of citation in the international search report.

3. The present application does not meet the criteria of Article 33(1) PCT, because **the subject-matter of claims 1-4 does not involve an inventive step** in the sense of Article 33(3) PCT.

There is a continuos need of improved enzymes having an industrial applicability. In particular, in D2 (considered as the closest prior art) hydantoin racemization is disclosed as the rate-determining step (see page 2, lines 48-50). D2 discloses the need for further hydantoin racemases having improved properties (see paragraphs [0006], [0007], and [0009]). In D2 a racemase having improved enzymatic properties was found see paragraph [0010]. Thus, the skilled person would have ben motivated to design screening processes to find new hydantoin racemases.

The problem to be solved by the present invention may therefore be regarded as to provide screening processes for hydantoin racemases. The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons.

4. The skilled person in view of the teachings of D1 and D2 and general common knowledge in enzymology would have designed an screening process where the hydantoin racemase is the limiting activity in order to easily detect racemases having improved properties.

4.1 **D1** discloses whole cell catalysts comprising the polynueclotide sequences encoding for hydantoinase hyuH, hydantoin racemase hyuA, and carbamoylase from Arthrobacter ausrescens DSM 3747. The hydantoinase hyuH is L-specific (see page 2, line 9 of D1), and thus enantioselective, **as referred to in claim 1 a) of the present application**.

4.2 The hydantoin racemase of D1 has a lower conversion rate compared to hydantoinase (**as referred to in claim 1 b) of the present application**). D1 discloses that the rates (conversion rates) of the 3 different enzymes is different, and if the rates are not in line wich each other, intermediates accumulate and less of the final desired product (the desired eniantomerically form of the amino acid) is obtained. In order to overcome this problem, the amout of hydantoinase, racemase, and carboamylase genes must be expressed according to their turnover rates (see page 3, last paragraph).

On pages 5 and 6, different ways of adjusting the expression of the three enzymes according to their turnover rates are disclosed. On pages 7 and 8 it is disclosed that the combination of plasmids pBW34 and PBW53 is most favourable, wherein the amount of overexpressed racemase and carbamoylase proteins is superior to that of hydantoinase. This means that the hydantoin racemase of D1 has a lower conversion rate compared to hydantoinase, since more copies of the racemase are required in order to have an improved production of the desired product.

4.3 The use of a chiral hydantoin having the opposite enantiomerically enriched form to the selectivity of the hydantoinase is the most obvious way to test the activity of the hydantoin racemase. As the hydantoinase of D1 is L-selective, it will not act on the D-hydantoin. In this way, if D-hydantoin is used as substrate, only the L-hydantoin isomerized by the racemase (or by the slower spontaneaous isomerization) will be converted by the L-selective hydantoinase.

Obviously if L-hydantoin would be used as a substrate, then the action of the hydantoin racemase will not be required in order to obtain the N-carboamyl-amino acid. Thus, in order to screen hydantoin racemases, the skilled person would be motivated to use a chiral hydantoin having the opposite enantiomerically enriched form to the selectivity of the hydantoinase

4.4 To measure the resulting N-carbamoyl-amino acid or the freed protons are two standard ways of measuring the activity of the enzyme.

Thus, the subject-matter of claim 1 is not considered to involve an inventive step.

5. Claims 2 and 3 do not involve an inventive step either. For example the hydantoinase of D3 (isolated form Arthrobacter cystallopoietes) is disclosed to have a wide substrate specificity including 5-monosubstituted hydantoin derivatives with aliphatic side chains (see abstract).

6. Claim 4 is not considered inventive either. The particular ratio of the constants of the hydantoinase to the racemase is among one of the possibilities the skilled person could contemplate without the involvement of inventive skill.

7. The subject-matter of claims 1 and 4 is unclear contravening the requirements of

Article 6 PCT. The screening process is apparently intended to identify new hydantoin racemases. According to claim 1b and claim 4, the racemase need to have a slower conversion rate than the hydantoinase. It appears contradictory that the conversion rate need to be known before the screening has been made.